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A single run, rapid polarity switching method for determination of 30 pharmaceuticals and personal care products in waste water using Q-Exactive Orbitrap high resolution accurate mass spectrometry

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Research Highlights

- SPE UPLC-Q-Exactive Orbitrap method was developed and validated for analysis of 30 PPCPs
- Rapid ESI polarity switching for analysis of acidic and basic analytes in one run
- Full scan MS mode allows for post-acquisition non-target screening
- First target/non-target report of PPCPs in waste/surface water from Egypt

Abstract

The analytical capability of the UPLC-Q Exactive™ Orbitrap MS was exploited for simultaneous determination of 30 acidic and basic PPCPs in a single run, using rapid polarity switching of the electrospray ionisation source. Full scan MS mode at resolution

of 35000 FWHM, Automatic gain control (AGC) target of 1E6 ions at injection time of 50 ms provided the optimum parameters for high sensitivity, together with sufficient data points per peak (≥15) for improved reproducibility. In addition to chromatographic retention times, method selectivity was achieved via applying high resolution accurate mass with low mass tolerance filter (<5 ppm) for identification of each target compound. Six-point linear calibration curves ($R^2 > 0.95$) were established for all target analytes over a concentration range of 1-1500 ng/ml. Good results were obtained for method accuracy (% recovery = 76–104%), inter- and intra-day precision (relative standard deviation <15%) at 3 concentration levels. Instrumental detection and quantification limits ranged from (0.02–1.21 ng/ml) and (0.07–4.05 ng/ml), respectively. While optimised MS/MS analysis through parallel reaction monitoring (PRM) mode provided slightly higher sensitivity, Full scan MS mode allowed for higher mass resolution (selectivity), more data points per peak (reproducibility) and more importantly, the potential for post-acquisition screening of non-target compounds. Following solid phase extraction (SPE) of target analytes, the method was successfully applied to provide first data on PPCPs occurrence in effluent and surface water samples (n=10) from Egypt. Moreover, screening for non-target compounds revealed the presence of bisphenol A, which was further confirmed via matching with an authentic standard. Overall, this study provides first insight into the high analytical capabilities of the Q-Exactive™ Orbitrap platform for both targeted/non-targeted analysis of PPCPs in environmental matrices.

Keywords: Q Exactive Orbitrap; Pharmaceuticals and personal care products; surface water; effluent; Egypt; non-target screening

Introduction

Pharmaceuticals and personal care products (PPCPs) represent a large group of chemicals with diverse structures and physicochemical properties. Pharmaceuticals include all prescription, over the counter and veterinary drugs used for prophylaxis or treatment of human or animal diseases, while personal care products are those applied mainly to improve the quality of daily life [1]. Pharmaceuticals are inherently potent, biologically-active chemicals, designed to achieve maximum bioavailability and prolonged duration of action in target organisms. The unintentional presence of a large number of PPCPs in the environment (water, sediment and biota) has received increasing scientific interest in the past few years. This has been mainly attributed to the inefficient removal of these chemicals during conventional waste water treatment processes [2, 3]. Other sources include the application of contaminated sewage sludge for fertilisation of soil and the leaching of agricultural run-off water to ground water reservoirs [2]. Currently, very little is known about the behaviour and fate of this large group of chemicals once released to the environment. This is of concern owing to the continuous input of PPCPs to the environment, together with the reported, persistence, bioaccumulative and toxic characters of various members of this group [1, 4, 5]. This is compounded by the current lack of knowledge on the impact of various PPCPs in the ecosystem on non-target organisms (e.g. fish, zooplankton, algae) [1].

The current status of PPCPs as emerging contaminants of high concern necessitates the development of sensitive, selective and accurate methods for determination of trace levels of these chemicals in various environmental compartments. Moreover, the large number of PPCPs requires the use of multi-residue, rapid and robust analytical methods to provide maximum possible information on the levels and profiles of these chemicals in each sample within reasonable time and cost of analysis [6]. To address this,

advanced methods using hyphenated liquid (LC) or gas chromatography (GC) and mass spectrometry (MS) techniques have been reported for analysis of PPCPs in the past few years [7, 8]. Due to their relatively hydrophilic nature, LC-MS is reported as the method of choice for multi-residue analysis of pharmaceuticals, endocrine disruptors, and illicit drugs, especially for thermally unstable chemicals [9]. Ultra-high pressure liquid chromatography (UPLC) displayed superior performance to conventional HPLC due to reduced particle size of the stationary phase. This results in enhanced resolution, narrower peaks and shorter run times [10]. Reversed phase (C_{18} or C_{18}-bonded phases) has been the most widely used mechanism of LC separation due to its versatility and capacity for separation of a broad range of compounds [8]. In terms of sample preparation, solid phase extraction (SPE) is the most popular and well-established technique for PPCPs in liquid environmental samples. Several SPE adsorbents with varying strength cation and/or anion exchange copolymers were reported to provide high extraction efficiency with low matrix-related interference for determination of different groups of PPCPs [10, 11].

However, rapid, sensitive and accurate multi-residue analysis of trace concentrations of PPCPs in complex environmental matrices continues to be a fascinating challenge. This is mainly driven by the continuous advance in analytical instrumentation leading to higher sensitivity, improved selectivity and wider dynamic range. The quadrupole (Q)-Orbitrap high resolution mass spectrometer (Q-Orbitrap/MS) is a good example of such advances. It provides mass resolution up to 280,000 full width at half maximum (FWHM), with acquisition speed of 12 Hz. This results in mass accuracy < 1 ppm and a linear dynamic range spanning 5 orders of magnitude. An important feature of the Q-Exactive Plus Orbitrap™ (Thermo Fisher Scientific, Bremen, Germany) is the rapid polarity switching between positive and negative ionisation modes in the heated

electrospray ionisation (HESI) source within a full cycle in <1 s. Moreover, the quadrupole adds another dimension by possible preselection of 10 precursor ions/scan. The selected precursor ions then undergo collision-induced fragmentation in parallel reaction monitoring mode (PRM) in order to conduct MS/MS analysis [12]. The cutting-edge technology of the Q-Exactive Orbitrap™ combined with the high separation power of UHPLC provides an excellent platform for analysis of complex chemical mixtures like PPCPs in environmental samples. To our knowledge, the analytical capability of this platform has yet to be fully explored for the purpose of PPCPs analysis. A survey of the literature revealed one analytical study using UHPLC-Q-Exactive Orbitrap™ for determination of 13 PPCPs in 2 runs for positively (12) and negatively (1) ionised compounds, separately [13]. This is similar to previously reported LC-MS/MS studies using other instruments, where 2 separate runs are required for multi-component analysis. In addition, the targeted MS/MS approach does not allow for retrospective screening approaches for identification of potential compounds of interest which were not targeted in the original analytical protocol [10, 14].

Therefore, the current study aims to exploit the full potential of the UHPLC-Q-Exactive Orbitrap™ platform for analysis of 30 PPCPs as representatives of different groups including: antibiotics, antiseptics, β-blockers, NSAIDs, narcotic analgesics, anti-hyperglycaemic agents, proton-pump inhibitors and insect-repellents (Table 1). Selection was based on priority pollutant lists developed by the European Union (EU) under the Water Framework Directive (WFD), as well as the United States Environmental Protection Agency (USEPA). Other selection criteria include: frequent environmental occurrence, persistence and toxicity to aquatic organisms [1]. The method aims to utilise the rapid polarity switching of the HESI source and the separation power of the UHPLC to achieve maximum separation in a single run within a

reasonably short analysis time. The developed method is validated and applied to provide insights into the levels and profiles of PPCPs in 10 effluent and surface water samples from Egypt. Furthermore, the advantage of acquiring full-scan high resolution MS data via the Orbitrap is explored for possible screening and identification of non-target compounds in the studied water samples.

Materials and Methods

Chemicals and Reagents

All solvents used in this study were purchased from Fisher Scientific™ (Loughborough, UK) and were of UPLC grade. Individual standards of 30 PPCPs (Table 1), in addition to isotope-labelled Caffeine-D9, Codeine-D3, Carbamazapine-D10, Estone-D4 and 4-Chlorophenol-2,3,5,6-D4 used as internal (surrogate) standards were purchased from Sigma-Aldrich™ (Irvine, UK) at the highest possible purity (>99 %). ^{13}C-tetrabromobisphenol A (^{13}C-TBBPA) and tris (2-chloroethyl) phosphate-D12 (TCEP-D12) used as recovery (syringe standards) were obtained from Wellington Laboratories (Guelph, ON, Canada). All standard stock solutions were prepared and further diluted in methanol. Oasis MCX and Oasis HLB cartridges (6 cm^3, 150 mg sorbent per cartridge) were obtained from Waters™ (Hertfordshire, UK). Ammonium formate (NH_4COOH), Na2EDTA, ammonium hydroxide (NH_4OH, 30 %), ammonium fluoride (NH_4F), Acetic acid and formic acid (HCOOH) were obtained from Sigma-Aldrich™ (Gillingham, UK). Nitrogen gas (oxygen free, 99.998%) was purchased from BOC (Birmingham, UK). Milli-Q water (Merck Millipore, Burlington, MA) was used for cleaning and sample preparation purposes.

Sampling

Water samples (1 L) were collected from the effluent of 5 waste water treatment plants (WWTPs) at Assiut governorate, Egypt. These include 3 major WWTPs in Assiut city (Al Helaly, Nazalat- Abdellah and El Walidiyaah), the water treatment plant of Sodfa town, in addition to the water treatment plant of Assiut University hospital (Figure SI-1). Furthermore, surface water samples were collected from the River Nile and El-Ebrahmiya canal in Assiut city. These were grab samples collected upstream of the WWTP discharge point in deactivated glass bottles and transferred immediately to the lab, where they were kept at 4 °C until extraction within 48 hours of collection.

Sample preparation

Individual and mixture stock solutions (0.5 g/L) of the targeted PPCPs (Table 1) were prepared in methanol and stored in dark amber vials at -20 °C. Working solutions were prepared fresh daily by diluting the stock solutions to the required final concentration and were stored at 4 °C for a maximum of 24 h. The isotope labelled internal standards were prepared and mixed separately at 1 ng/µL in methanol and kept in dark amber vials at -20 °C.

Environmental water samples were extracted by solid phase extraction (SPE) using Oasis MCX 6 ml cartridges and Waters™ 20-port controlled pressure vacuum manifold equipped with 50Hz vacuum pump (Waters, Hertfordshire, UK). The SPE cartridges were pre-conditioned with 3 mL of methanol following by 3 mL of Milli-Q water. 250 mL of the water sample were spiked with 100 ng of isotopically-labelled internal standards mixture and treated with 500 mg Na_2EDTA to release the free form of target analytes (e.g. Doxycyclin) from potential complexes with Ca^{2+} and Mg^{2+} in environmental waters [15]. The samples were loaded onto the pre-conditioned cartridges at a flow rate of 5 mL/min. The cartridges were washed with 3 mL of 0.5 % HCOOH in Milli-Q water (3

mL/min). After drying, PPCPs were eluted with 5 mL of methanol following by 5 mL of 5 % NH_4OH in methanol. The combined eluate was dried under a gentle stream of nitrogen using a TurboVap II® evaporator (Biotage™, Sweden) and reconstituted in 100 µL of methanol containing 25 pg/µL of ^{13}C-TBBPA and TCEP-D12 used as recovery (syringe) standards for QA/QC purposes.

Instrument Analysis

Samples were analysed on a UPLC-Q Exactive Orbitrap-HRMS system (Thermo Fisher Scientific™, Bremen, Germany) composed of a Dionex Ultimate 3000 liquid chromatograph equipped with a HPG-3400RS dual pump, a TCC-3000 column oven and a WPS-3000 auto sampler. The UPLC is coupled to a Q-Exactive Plus Orbitrap mass spectrometer equipped with a heated electrospray ionisation (HESI) ion source. Chromatographic separation was achieved on an Accucore RP-MS column (100 x 2.1 mm, 2.6 µm) with 2 mM NH_4COOH/2mM NH_4F in water (mobile phase A) and 0.5 % acetic acid in methanol (mobile phase B). A gradient method at 400 µL/min flow rate was applied as follows: start at 2 % B, stay for 1 min; increase to 98 % B over 11 min, held for 1 min; then decrease to 2 % B over 0.1 min; maintained constant for a total run time of 16 min. Injection volume was 5 µL. The Orbitrap parameters were set as follows: alternate switching (-)/(+) ESI full scan mode, sheath gas flow rate 20 AU, discharge volage 4.5 kV, capillary temperature 320 °C, resolution 35000 FWHM, AGC target $1E^6$, maximum injection time (IT, the maximum time allowed to obtain the set AGC target) 50 ms and scan range 125 to 750 m/z. MS/MS analysis using PRM was also attempted. The chromatographic and Orbitrap MS parameters were the same as in full scan acquisition, except for: AGC target $2E^5$, maximum IT 100 ms and resolution 17,500 FWHM).

Method Validation and quantification

Method linearity was investigated via triplicate injections of 6 point calibration curve for each of the studied analytes over a concentration range of 1 – 1500 ng/mL, using a fixed concentration of 100 ng/mL of the isotope labelled IS. Linearity was evaluated through the linearity coefficients (R^2) of the obtained calibration curves.

Other method validation parameters were calculated using Milli-Q water spiked with the target PPCPs at 3 concentration levels (10, 250 and 750 ng/mL).

Accuracy was estimated as the percentage recovery of target analytes and evaluated through the percent deviation from the known spiked concentration level.

Precision was calculated as relative standard deviation (RSD %) for inter- and intra-day multiple injections. Nine injections covering 3 concentration levels (3 injections each) were used for assessment of precision. Further validation of method precision was performed via calculation of the RSD % for triplicate analyses of 3 different samples (tapwater spiked with 500 ng/L of all target PPCPs, surface water from the River Nile (2G) and effluent sample (1A)). The tapwater was collected in the lab and allowed to stand for 30 min for evaporation of Cl_2 prior to further processing.

Limit of detection (LOD) and limit of quantification (LOQ) were estimated using the signal to noise (S:N) approach. Instrumental detection limit (IDL) was calculated as the lowest concentration that gives a S:N ratio of 3:1, while Instrumental quantification limit (IQL) was calculated as the lowest concentration that gives a S:N ratio of 10:1. Method quantification limits (MQL) were determined by repeated injection of tapwater samples spiked at low concentrations of target compounds. The concentration that produces a S:N ratio of 10 was estimated as the MQL.

Quality assurance/quality control (QA/QC)

None of the target compounds were detected in method blanks (one blank for every 5 samples; each blank is composed of 250 mL Milli-Q water treated like a sample). Therefore, no blank correction of the results was required.

Recoveries of the isotope-labelled internal standards were calculated against the syringe standards in all environmental and QA/QC samples. High percent recoveries (>70 %) of all five internal standards were obtained indicating good overall performance of the method.

A calibration standard containing all the target compounds and IS (500 ng/mL) was injected before and after each sample batch (Figure 1). For a given peak to be identified as a target analyte in a sample; the relative retention time (RRT) of the peak in the sample must be within ± 0.1 min of the average value determined for the same analyte in the 2 calibration standards ran before and after that sample batch.

Results and Discussion

Method Optimisation

Solid phase extraction was documented by several authors as the method of choice for PPCPs in wastewater samples using various sorbent beds [8]. In the current study, we tested two of the most widely reported sorbent beds for extraction of various PPCPs, namely: Oasis MCX and Oasis HLB. A paired t-test for comparison of means revealed no significant differences between the recoveries of target analytes in spiked tap water samples (500 ng/L of all target PPCPs, n=3) from both solid phases (Figure SI-2). However, it was generally observed that a higher chromatographic baseline and more spectral interference occurred in real effluent samples extracted with HLB cartridges compared to MCX (Figure 2). This is in agreement with the results of Petrie et al. [6] and can be attributed to the non-selective nature of the hydrophilic-lipophilic balanced,

reversed-phase HLB sorbent bed, which can cause significant matrix-related interferences when using ESI mode [16]. Therefore, Oasis MCX, which is built upon the HLB copolymer with mixed mode cation-exchange and reversed phase interactions, was applied for extraction of all our samples.

Although baseline chromatographic separation of all analytes was not targeted due to subsequent MS analysis (Figure 1), the method was optimised towards achieving a better peak shape and higher intensity in subsequent ESI ionisation. Therefore, a simple mobile phase gradient based on H_2O and methanol was chosen due to the observed overall reduction of ESI signal intensity when using acetonitrile compared to methanol. This may be attributed to the reduced charge status of ionised species in the electrospray droplets by the neutral vapour of acetonitrile in the atmospheric region of the source [17]. Acetic acid buffer had a substantial effect on enhancing the peak shape and signal intensity of basic analytes (Figure SI-3) via promoting their protonation in ESI positive mode [18]. Moreover, the use of NH_4F as a mobile phase additive resulted in significant enhancement of signal intensity for the steroid hormones 17α-ethinylestradiol and β-estradiol by 360 % and 480 %, respectively. Petrie et al. reported more than 400 % increase in the signal intensity for the steroid hormones E1 and E2 upon using NH_4F as a mobile phase additive for LC-ESI(-ve)-MS/MS analysis [6]. Similarly, Carmona et al. reported NH_4F to improve the peak shape and signal intensity compared to ammonium formate for LC-ESI(-ve)-MS/MS analysis of various PPCPs including indomethacin, ibuprofen, diclofenac and gemfibrozil [19]. This may be explained by the strong electronegativity of the $[F]^-$ anion, which enhances deprotonation of the acidic analytes in ESI negative mode [19]. In the current study, NH_4F was used mainly due to significant enhancement of the signals for steroid

hormones, while its influence on other acidic analytes (e.g. ibuprofen, diclofenac and gemfibrozil) was less evident (Figure SI-4).

Several mass spectrometric parameters were optimised to maximise method sensitivity and achieve the highest signal/noise (S/N) ratio for the studied compounds. While the Q-Exactive Orbitrap™ enables very high mass resolution (up to 280,000 FWHM), the scan (dwell) time increases with increasing mass resolution. Long dwell time per scan cycle results in broad chromatographic peaks due to few data points acquired per each peak as it elutes from the column. This ultimately leads to reduced overall sensitivity and quantitative reproducibility of the analytical method. Therefore, a minimum of 8-10 data points across an LC peak is required to define its shape and enable reproducible quantitation based on area under the peak, while an optimum of 15-20 points are required to expose subtle peak-shape features [20]. Another unique feature of the Orbitrap MS is the automatic gain control (AGC), which defines the maximum number of ions (from 2×10^4 - 4×10^6) to be injected into the mass analyser within a specified injection time (IT). To optimise for these multiple parameters, we adopted a systemic approach for each target analyte by studying the concomitant impact of mass resolution (up to 280,000 FWHM) and AGC (up to 4×10^6) on the peak area of the studied compound (Figure 3) with defined IT of 50 milliseconds and a minimum of 15 data points per peak. Despite few non-significant variations for a few compounds, results revealed the optimum MS parameters for the overall method as: resolution = 35000 FWHM, AGC target = 1×10^6 ions and IT = 50 ms.

Method selectivity and minimisation of potential interferences from co-extracted molecules in real samples were achieved via monitoring the molecular ion peak for each of the target compounds using its specified accurate mass (Table 1) with the following

filters applied: maximum mass tolerance of 5 ppm, retention time window of 20 seconds and relative retention time (to the designated labelled IS) window of 5 seconds. The extracted ion chromatograms (EIC) according to these filters showed well-defined correctly identified and appropriately integrated peaks in the studied real samples.

Method Validation

Calibration curves showed good linearity of the method over a wide concentration range (1-1500 ng/mL) for all the studied PPCPs with a mass error <5 ppm at 35000 FWHM. The linearity coefficient (R^2) exceeded 0.99 for all target PPCPs except for 6 analytes where it ranged from 0.95 – 0.98 (Table 2). Average percent recoveries of all analytes ranged from spiked Milli-Q water samples ranged between 76-104 % at 3 concentration levels (Table 2) with no statistically significant difference between the recoveries of each analyte at the different concentration levels (Table SI-2), indicating good accuracy of the method. Potential matrix effects were investigated via triplicate analysis of spiked tap water (500 ng/L of all target PPCPs) with recoveries ranging from 78 – 106 % (Table SI-3). Furthermore, an effluent sample (1A) and a surface water sample (2G) (Figure SI-1) were spiked with 500 ng/L of oxazepam (ionizes in ESI positive mode) and 17α-ethinylestradiol (ESI negative), which were not detected in these 2 water samples. Good recoveries of 103 ± 8 % and 78 ± 11 % were obtained for oxazepam and 17α-ethinylestradiol, respectively. However, the difference between the average recovery of oxazepam from real water samples (103 ± 8 %) and that from spiked tap water (92 ± 8 %) may indicate potential matrix-related effects, which have been previously reported with ESI ionisation [15]. Evaluation of matrix suppression of the ESI signal for target analytes was performed using the matrix-matched calibration method described by Kasprzyk-Hordern et al. (2008)[21]. Results revealed higher matrix suppression of the studied PPCPs in effluent (7 – 49%) compared to surface

water (5 – 44%) (Table SI6). Full evaluation of the extent of matrix effects and their influence on the accuracy of determination of various PPCPs in environmental waters is difficult due to existing levels of PPCPs in real water samples as a result of their ubiquitous status in the aquatic environment [1]. Therefore, inter-laboratory studies and development of certified reference materials for PPCPs in environmental water samples is highly recommended.

Investigation of the method inter- and intra-day repeatability at 3 concentration levels revealed RSD values <15 % for all the studied compounds (Table 2). In addition, triplicate analysis of spiked tapwater, effluent (1A) and surface water (2G) samples also showed RSD values <15 % indicating good precision of the developed method (Table SI-4).

Method sensitivity is demonstrated by the limits of detection and quantification (Table 2). Our method quantification limits ranged from 2 - 84 ng/L in tapwater, which is in-line with previously reported UPLC-MS/MS methods for analysis of PPCPs in environmental water [10]. Combined with the inherent advantages of rapid ESI-polarity switching, high mass resolution (35000 FWHM) and low mass tolerance (< 5 ppm) of the Orbitrap, this makes the developed method suitable for determination of trace level PPCPs in environmental water samples (e.g. surface water).

Full Scan vs MS/MS analysis using the Q-Exactive Orbitrap™

The Q-Exactive Orbitrap™ used in this study is equipped with a quadrupole filter that allows preselection of 10 precursor ions to undergo collision induced dissociation (CID), prior to high resolution mass scanning of the produced fragments in the Orbitrap analyser (i.e. MS/MS analysis). It is generally perceived that MS/MS analysis provides higher sensitivity than MS methods by minimising baseline interferences resulting in higher S/N ratios [22]. However, this is largely accepted when comparing the results of

tandem MS/MS analysis to single, low mass resolution MS but has not been fully evaluated for the high resolution Orbitrap MS applied in the current study. Therefore, we considered MS/MS analysis of our target PPCPs to exploit the full potential of the instrument. Since only 10 precursor ions can be selected, the acquisition method was divided into several time windows to monitor our 30 analytes and 5 istotope-labelled internal standards. This challenges the method capacity for analysis of a large number of compounds due to limited number of precursor ions in each time window. Furthermore, it is generally recommended to allow a tolerance margin of ±0.5 min for analyte retention times in each time window to account for slight changes in retention times due to sample matrix effects and column aging [13]. However, this was not possible in our method due to the large number of compounds eluting at close retention times between 7.97 to 11.54 min (Table 1) resulting in "narrow" time windows (±0.3 min), which reduces the overall robustness of the method. As reported previously [13], the use of the quadrupole increases the time of the scan cycle, which requires decreasing the orbitrap mass resolution to 17500 FWHM and the AGC target to $2x10^{-5}$ to obtain sufficient number of data points (>10) across each peak.

The precursor ions, major fragment ions, optimised collision energy and estimated IQLs for all target PPCPs, obtained from injection of standard solutions are provided in Table SI-5. However, it is evident that there is no substantial improvement of sensitivity in the MS/MS mode compared to the full scan mode to mitigate for the reduced method robustness, AGC target and mass resolution associated with the MS/MS mode in this instrument. This may be attributed to the high resolution (35000 FWHM) and low mass tolerance (<5 ppm) enabled by the Orbitrap in full scan mode, which reduces potential co-eluted interferences resulting in a low baseline and high S/N ratio.

The rapid, high resolution full scan MS analysis enabled by the Orbitrap provides another major advantage, as it enables post-acquisition independent data analysis. This allows screening for non-target compounds in the studied samples, which is beneficial to PPCPs monitoring in environmental samples due to the great diversity of this contaminant class. To illustrate, post-acquisition screening of our effluent and surface water samples revealed the potential presence of bisphenol A, which is a common environmental contaminant that was not targeted in our method. The identity of bisphenol A was then confirmed via comparison of its accurate mass, retention time and molecular ion cluster to an authentic chemical standard. Further confirmation of bisphenol A identity was achieved via sample fortification with the authentic standard resulting in boosting the area of the designated chromatographic peak (Figure 4). Investigation of our method blanks revealed the detection of Bisphenol A in 3 out 8 blanks with peak areas less than 5% of those detected in the real water samples. While this does not allow for accurate quantification of bisphenol A in our samples, it demonstrates the potential for combined target/non-target approaches for PPCPs analysis using the high resolution, full scan mode of the Orbitrap.

Method Application

The developed method was applied for simultaneous determination of 30 target PPCPs in 5 effluent and 5 surface water samples collected from Assiut Governorate, Egypt. Apart from the anti-malarial compounds mefloquine HCl and DEET, all target PPCPs were successfully, detected and quantified in at least one of the studied samples (Table 3). The method showed good robustness with real samples, with none of the compounds shifting outside its specified retention time window. The applied high resolution and low mass tolerance resulted in low chromatographic baselines and well-defined peaks, which is advantageous for accurate integration of peak areas by the

Xcalibur™ software. To our knowledge, this is the first report of PPCPs in environmental water samples from Egypt. Apart from the anti-malarial drugs Mefloquine and DEET, all target PPCPs were detected and quantified with high mass accuracy (<5 ppm) at high mass resolution (35000 FWHM) in one or more of the studied water samples. Our results show high levels of acetaminophen (144-16000 ng/L), ibuprofen (26-6700 ng/L) and glyburide (253-4150 ng/L), which were detected in all samples. This is generally consistent with concentrations of these compounds reported in effluent and surface water samples from South Africa [23] and Nigeria [24]. However, it should be noted that PPCPs levels in water are largely dependent on the usage patterns and prescription frequencies in the studied area and may vary significantly between different countries or geographical areas [1]. Therefore, the absence of anti-malarial drugs in the studied water samples may be attributed to the very low incidence of malaria in Egypt compared to west and south African countries [25].

Conclusion

A sensitive, rapid and robust UPLC-Q-Exactive™ Orbitrap high resolution MS method was developed and validated for mutli-residue analysis of 30 PPCPs. The method applies rapid polarity switching in the heated ESI source for simultaneous analysis of positive and negative ionised compounds in one chromatographic run of 16 min. The method's high resolution (35000 FWHM) and low mass tolerance (<5 ppm) minimises potential interference from co-eluted compounds and provides a low chromatographic base-line, leading to high S/N ratios in extracted ion chromatograms of the target compounds. This resulted in high sensitivity of the full scan MS method comparable to the performance of MS/MS analysis. Moreover, full scan MS analysis provides the added advantage of post-acquisition screening for non-target compounds. The method was applied successfully to provide the first data on levels of target PPCPs in effluent and

surface water samples from Assiut, Egypt. Post-acquisition screening for non-target compounds revealed the presence of bisphenol A, which was further confirmed via matching with an authentic standard. Overall, this work demonstrates the high analytical capabilities of the Q-Exactive™ Orbitrap and provides the first insight into the potential of this platform for both targeted/non-targeted analysis of PPCPs in environmental matrices.

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Supplementary Material

Full details of the studied PPCPs physico-chemical properties, method performance/validation parameters and parallel reaction monitoring (MS/MS) optimisation are provided as supplementary material.

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Figures

Figure 1: Reconstructed UPLC-Q-Exactive™ Orbitrap/MS chromatogram of target PPCPs and IS (500 ng/mL in methanol).

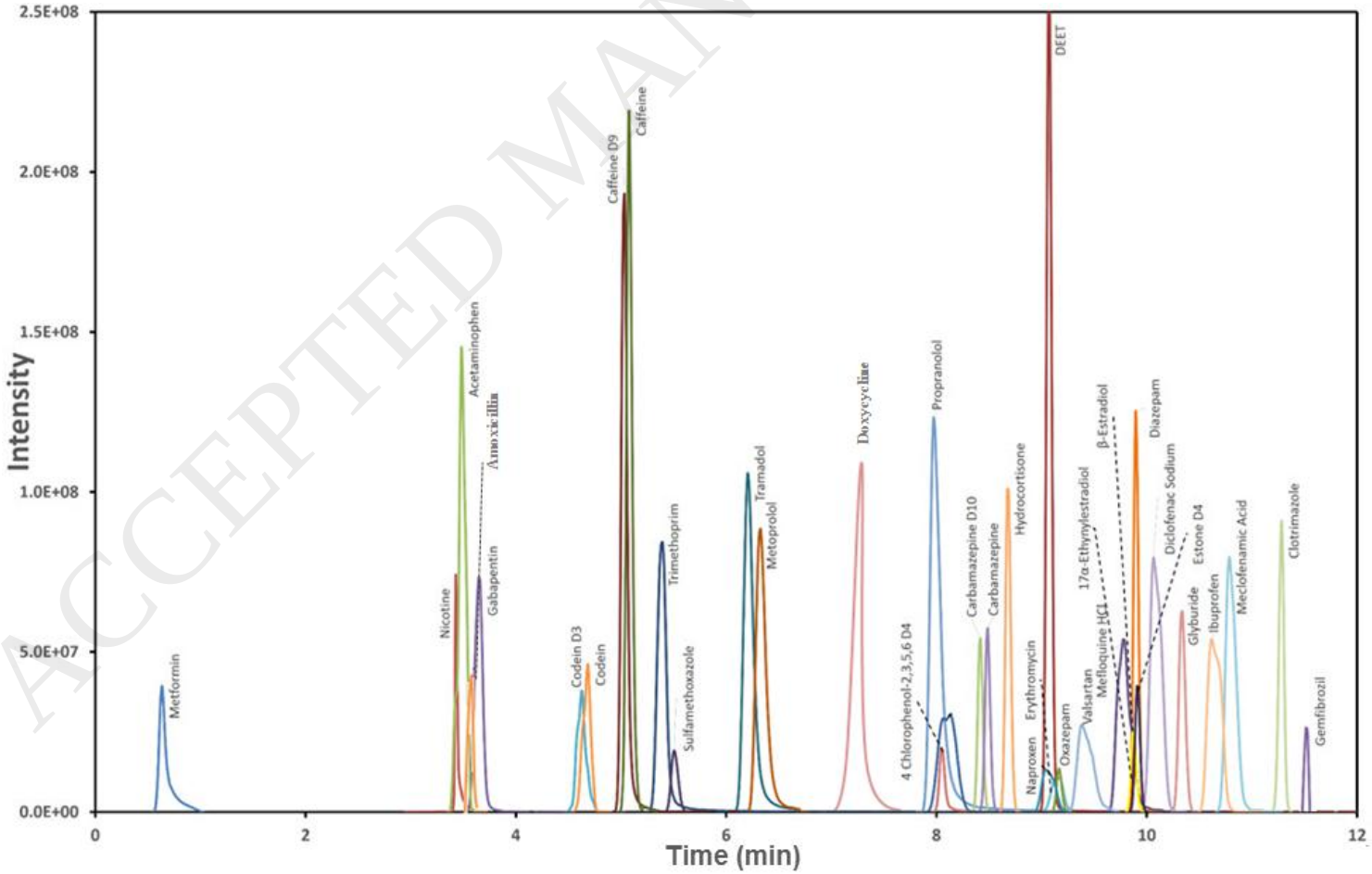


Figure 2: Total ion chromatogram of effluent sample 1A following SPE on (a) OASIS HLB crtridge (higher baseline) and (b) Oasis MCX (lower baseline). Inset shows the extracted ion chromatogram for Diazepam at m/z = 285.07928 (representative example) with a higher baseline in the Oasis HLB sample.

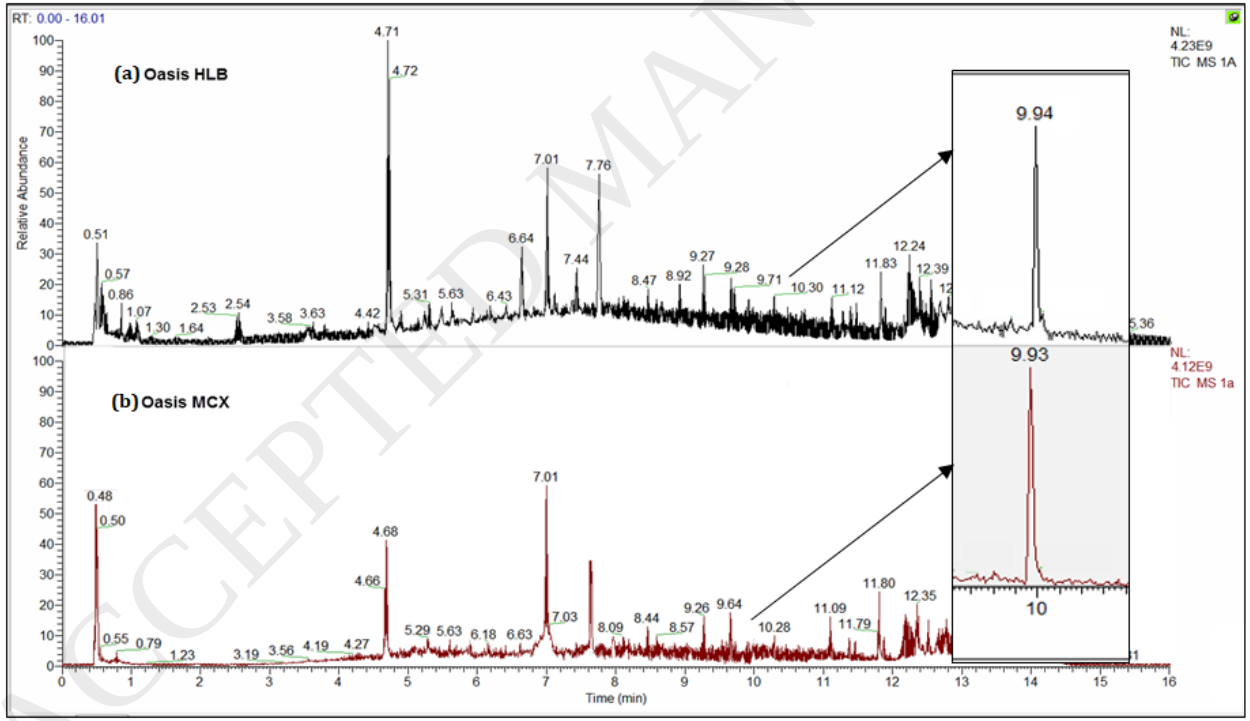


Figure 3: Representative example showing the impact of mass resolution (FWHM), Automatic gain control target (ions) on the peak are of Nicotine (750 ng/mL) and the number of data points per selected peak.

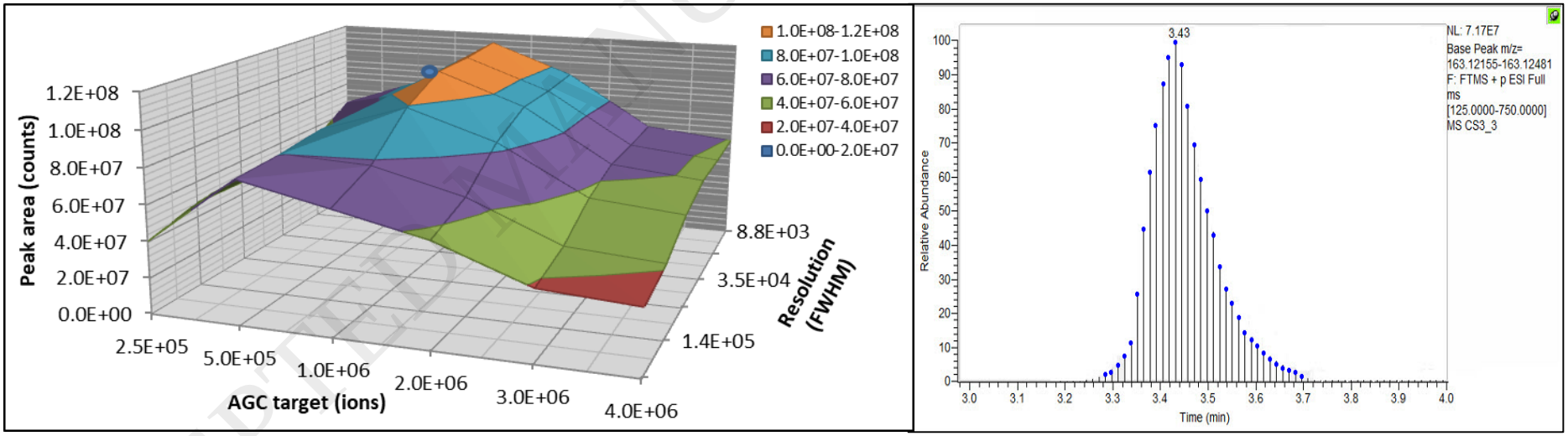
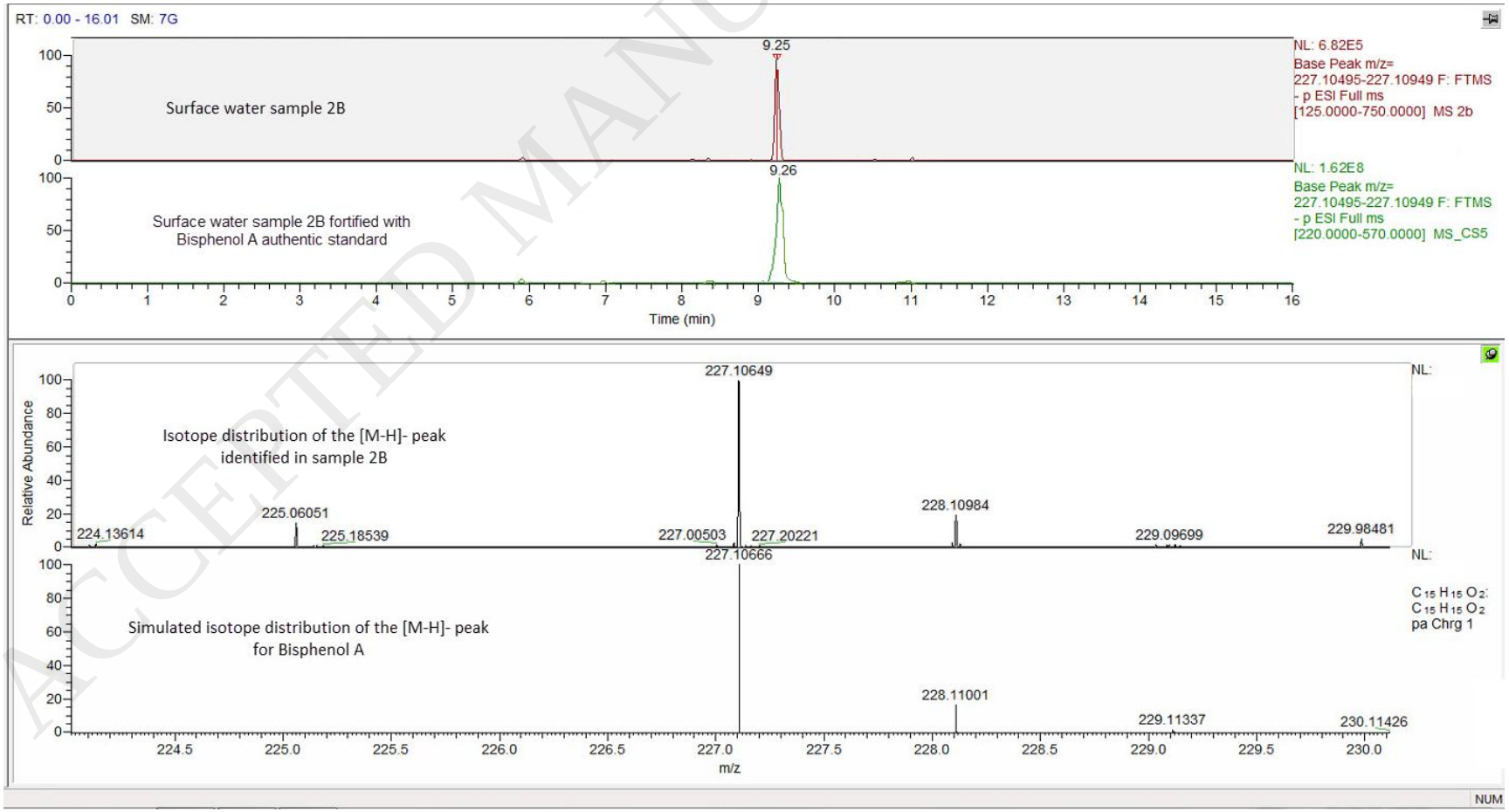


Figure 4: Post-acquisition identification of non-targeted Bisphenol A in the studied water samples through its accurate mass, isotope cluster and confirmation by fortification with Bisphenol A standard.



Tables

Table 1: List of target PPCPs and their chemical formula, accurate mass, ESI mode, retention time (t_R), as well as the internal (surrogate) standards used for quantification.

Name	Therapeutic group	Chemical formula	Ionisation	Mass (Da)	t_R (min)	Internal standard
Metformin	Anti-diabetic	$C_4H_{11}N_5$	+ve	130.10884	0.64	Codeine-D3 (t_R= 4.63 min)
Nicotine	Stimulant	$C_{10}H_{14}N_2$	+ve	163.12318	3.43	Codeine-D3
Acetaminophen	Analgesic	$C_8H_9NO_2$	+ve	152.07143	3.46	Codeine-D3
Amoxicillin	Antibiotic	$C_{16}H_{19}N_3O_5S$	+ve	366.09687	3.53	Codeine-D3
Gabapentin	Anti-convulsant	$C_9H_{17}NO_2$	+ve	172.13417	3.65	Codeine-D3
Codeine	Narcotic analgesic	$C_{18}H_{21}NO_3$	+ve	300.16089	4.69	Codeine-D3
Caffeine	Stimulant	$C_8H_{10}N_4O_2$	+ve	195.08862	5.17	Caffeine-D9 (t_R= 5.13 min)
Trimethoprim	Anti-bacterial	$C_{14}H_{18}N_4O_3$	+ve	291.14540	5.40	Codeine-D3
Sulfamethoxazole	Anti-bacterial	$C_{10}H_{11}N_3O_3S$	+ve	254.05949	5.50	Caffeine-D9
Tramadol	Narcotic analgesic	$C_{16}H_{25}NO_2$	+ve	264.19584	6.20	Codeine-D3
Metoprolol	Beta-blocker	$C_{15}H_{25}NO_3$	+ve	268.19076	6.33	Codeine-D3
Doxycycline	Antibiotic	$C_{22}H_{24}N_2O_8$	+ve	445.14963	7.47	Codeine-D3
Propranolol	Beta-blocker	$C_{16}H_{21}NO_2$	+ve	260.16433	7.97	Codeine-D3
Carbamazepine	Anti-convulsant	$C_{15}H_{12}N_2O$	+ve	237.10333	8.49	Carbamazepine-D10 (t_R= 8.49 min)
Hydrocortisone	Steroid	$C_{21}H_{30}O_5$	+ve	363.21686	8.67	Carbamazepine-D10
Naproxen	NSAID	$C_{14}H_{14}O_3$	-ve	229.08824	9.05	4 Chlorophenol-D4 (t_R= 8.05 min)
DEET	insect repellant	$C_{12}H_{17}NO$	+ve	192.13931	9.07	Carbamazepine-D10
Erythromycin	Antibiotic	$C_{37}H_{67}NO_{13}$	+ve	734.47192	9.14	Carbamazepine-D10

Oxazepam	Sedative, hypnotic	$C_{15}H_{11}ClN_2O_2$	+ve	287.05860	9.17	Carbamazepine-D10
Valsartan	Anti-hypertensive	$C_{24}H_{29}N_5O_3$	-ve	434.22117	9.56	4 Chlorophenol-D4
Mefloquine	Anti-malarial	$C_{17}H_{16}F_6N_2O$	+ve	379.12231	9.78	Carbamazepine-D10
17α-ethynylestradiol	Steroid	$C_{20}H_{24}O_2$	-ve	295.17047	9.87	Estone-D4 (t_R= 9.91 min)
β-estradiol	Steroid	$C_{18}H_{24}O_2$	-ve	271.16998	9.88	Estone-D4
Diazepam	Sedative, hypnotic	$C_{16}H_{13}ClN_2O$	+ve	285.07928	9.89	Carbamazepine-D10
Diclofenac Na	NSAID	$C_{14}H_{10}Cl_2NNaO_2$	-ve	294.01031	10.06	4 Chlorophenol-D4
Glyburide	Anti-diabetic	$C_{23}H_{28}ClN_3O_5S$	-ve	492.13818	10.34	4 Chlorophenol-D4
Ibuprofen	NSAID	$C_{13}H_{18}O_2$	-ve	205.12297	10.61	4 Chlorophenol-D4
Meclofenamic acid	NSAID	$C_{14}H_{11}Cl_2NO_2$	-ve	294.01031	10.78	4 Chlorophenol-D4
Clotrimazole	Anti-fungal	$C_{22}H_{17}ClN_2$	+ve	345.11676	11.28	Carbamazepine D10
Gemfibrozil	Anti-hyperlipidemic	$C_{15}H_{22}O_3$	-ve	249.15001	11.54	4 Chlorophenol-D4

Table 2: Summary of method validation parameters.

Name	R^{2*}	Accuracy[#] (% Recovery ±SD)	Precision (RSD %)[$]					IDL (ng/ml)	IQL (ng/ml)	MQL† (ng/L)
			Intra-day**	Inter-day**	Tap water[&]	Effluent (1A)[‡]	Surface water (2G)[‡]			
Metformin	0.9972	93.2 ± 6.1	4.7	6.6	6.8	9.3	10.9	0.10	0.33	9.5
Nicotine	0.999	92.2 ± 5.8	4.1	6.4	5.6	11.6	6.9	0.50	1.67	13.3
Acetaminophen	0.9938	96.4 ± 4.5	3.3	4.7	2.1	5.1	5.9	0.10	0.33	2.8
Amoxicillin	0.9924	88.6 ± 3.1	2.1	3.5	3.8	12.2	7.4	1.10	3.67	22.4
Gabapentin	0.9951	90.4 ± 5.8	7.7	6.4	9.5	<MQL	9.9	0.28	0.93	5.2
Codeine	0.9984	92.0 ± 3.1	2.7	3.4	6.0	<MQL	9.7	0.23	0.77	5.0
Caffeine	0.9951	101.4 ± 4.9	4.1	4.8	8.2	5.4	8.3	0.80	2.80	7.2
Trimethoprim	0.9975	96.3 ± 4.1	3.6	4.3	1.5	11.2	7.6	0.04	0.12	2.4
Sulfamethoxazole	0.9957	92.8 ± 3.0	2.4	3.2	4.9	13.4	3.3	0.06	0.20	3.4
Tramadol	0.9958	91.8 ± 4.0	3.5	4.3	4.2	10.8	9.9	0.17	0.56	4.6
Metoprolol	0.9992	93.1 ± 3.3	2.0	3.5	4.8	<MQL	<MQL	0.02	0.07	2.7
Propranolol	0.9957	95.6 ± 5.8	6.7	6.5	8.0	15.1	11.9	0.04	0.14	4.7
Doxycycline	0.9979	85.7 ± 4.1	4.6	4.8	4.0	<MQL	<MQL	0.24	0.79	22.9
Carbamazepine	0.9749	88.2 ± 3.2	3.9	3.6	5.2	16.3	13.8	0.02	0.07	2.5
Hydrocortisone	0.9856	83.5 ± 4.8	5.1	5.8	6.2	12.6	7.5	0.34	1.13	37.8
Naproxen	0.9629	90.2 ± 4.7	3.4	5.2	5.4	<MQL	<MQL	0.09	0.30	4.7
DEET	0.9524	94.1 ± 4.4	4.5	4.9	3.6	<MQL	<MQL	0.11	0.37	5.7
Erythromycin	0.9920	83.5 ± 3.7	3.2	4.5	5.1	8.5	<MQL	0.25	0.84	22.0
Oxazepam	0.9923	94.7 ± 5.3	6.3	5.6	3.4	<MQL	<MQL	0.15	0.49	6.3
Valsartan	0.9951	92.5 ± 7.3	9.1	9.9	4.5	10.3	8.2	0.32	1.05	8.6
Mefloquine	0.9937	86.7 ± 4.5	6.3	5.2	6.0	<MQL	12.1	0.30	0.99	24.7
17α-ethynylestradiol	0.9952	78.9 ± 5.0	5.3	6.4	6.3	<MQL	<MQL	1.21	4.05	83.8
β-estradiol	0.9951	76.9 ± 5.2	4.6	7.6	4.9	<MQL	<MQL	1.16	3.87	81.0

Diazepam	0.9739	94.9	±	6.8	4.7	7.2	6.3	<MQL	<MQL	0.13	0.43	4.7
Diclofenac Sodium	0.9944	89.4	±	3.9	4.9	4.3	4.7	8.9	6.9	0.15	0.50	9.8
Glyburide	0.9951	88.3	±	4.5	4.2	5.1	6.2	3.5	3.6	0.30	0.99	12.9
Ibuprofen	0.9949	90.9	±	3.3	3.5	3.7	8.7	11.3	<MQL	0.12	0.41	8.9
Meclofenamic acid	0.9994	86.1	±	4.7	3.9	5.5	8.8	6.3	6.8	0.17	0.57	10.3
Clotrimazole	0.9619	101.8	±	4.0	3.7	3.9	6.9	13.5	8.9	0.36	1.19	16.3
Gemfibrozil	0.9906	92.3	±	6.6	7.4	7.2	8.7	<MQL	9.6	0.31	1.05	14.5

* Linearity co-efficient over a range of 1-1500 ng/ml.

Recovery % of triplicate measurements at 3 spiked concentration levels (10, 250 and 750 ng/ml) in Milli-Q water. Details at each level are provided in the SI section.

$ Relative standard deviation (RSD%) of triplicate measurements.

** RSD% of triplicate measurements at 3 spiked concentration levels (10, 250 and 750 ng/ml) in Milli-Q water. Details at each level are provided in the SI section.

& RSD% of triplicate analysis of tapwater samples spiked with 500 ng/L of all target PPCPs.

‡ RSD% of triplicate analysis of un-spiked samples.

† MQL determined in spiked tapwater.

Table 3: Concentrations (ng/L) of target PPCPs in the studied effluent and surface water samples.

Name	Effluent samples					Surface water samples				
	1A	1B	1C	1D	1E	2F	2G	2H	2I	2J
Nicotine	365	736	567	835	419	116	90	269	378	98
Metformin	219	589	5613	1109	168	32	63	23	21	36
Acetaminophen	1509	978	15947	3042	1582	954	144	207	392	776
Codein	63	<MQL	466	29	<MQL	<MQL	18	14	21	15
Amoxicilin	<MQL	129	2038	<MQL	29	<MQL	24	<MQL	<MQL	28
Gabapentin	<MQL	40	279	<MQL	<MQL	<MQL	8	<MQL	12	<MQL
Trimethoprim	1060	271	2738	459	650	230	116	210	224	175
Caffeine	84	1739	855	121	70	12	41	15	7	54
Tramadol	353	508	1103	192	282	41	93	56	32	58
Metoprolol	34	218	1089	67	57	17	8	5	9	12
Sulfamethoxazole	<MQL	<MQL	19	<MQl	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Propranolol	8	19	187	62	<MQL	<MQL	6	<MQL	7	<MQL
Erythomycin	52	<MQL	275	106	<MQL	<MQL	<MQL	<MQL	33	61
Carbamazepine	63	151	342	<MQL	<MQL	<MQL	6	<MQL	8	1
Hydrocortizone	43	83	128	77	46	36	43	64	42	40
Mefloquine	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
DEET	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Oxazepam	<MQL	<MQL	39	<MQL	10	<MQL	<MQL	<MQL	<MQL	<MQL
Doxycycline	<MQL	<MQL	29	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Clotrimazole	31	<MQL	231	<MQL	43	<MQL	23	<MQL	18	<MQL
Naproxen	<MQL	29	89	<MQL	13	<MQL	6	<MQL	<MQL	8
Diazepam	<MQL	17	58	<MQL	<MQL	<MQL	<MQL	<MQL	9	<MQL
β-estradiol	<MQL	<MQL	165	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
17α-ethynylestradiol	<MQL	<MQL	219	<MQL	104	<MQL	<MQL	<MQL	<MQL	<MQL

Valsartan	107	258	594	318	290	63	55	104	59	36
Glyburide	2120	798	4162	550	1438	333	628	393	365	253
Diclofenac Sodium	269	79	3614	172	201	35	<MQL	77	44	<MQL
Ibuprofen	1497	1661	6702	812	1092	51	26	91	62	34
Meclofenamic acid	17	<MQL	52	<MQL	<MQL	<MQL	12	<MQL	<MQL	<MQL
Gemfibrozil	<MQL	<MQL	105	44	<MQL	<MQL	17	<MQL	16	21